# 14 THC AND THC-CARBOXYLIC ACID CONFIRMATION IN BLOOD BY DPX EXTRACTION AND GCMS ANALYSIS

## 14.1 Summary

14.1.1 Delta-9-tetrahydrocannabinol (THC), principal metabolite 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCA) and their deuterated internal standards are extracted and analyzed in biological samples using an acetonitrile precipitation. After centrifugation, the supernatant acetonitrile layer is made acidic and extracted with Disposable Pipette Extraction (DPX) tips. The DPX tips contain solid phase extraction (SPE) powder. The THC and THCA are adsorbed on to the SPE powder and extracted with Hexane/Ethyl Acetate. The extracted solvent is dried under nitrogen and derivatized with N,O-bis-(Trimethylsilyl)trifluoroacetamide (BSTFA) to form the trimethylsilyl derivatives of THC, THCA, deuterated THC, and deuterated THCA. The derivatized samples are injected into the GC/MS for confirmation by selected ion monitoring (SIM).

## 14.2 Specimen Requirements

14.2.1 1.0 mL of blood, biological fluid or tissue homogenate.

## 14.3 Reagents And Standards

14.3.1 Delta 9-THC, 1 mg/mL

14.3.2 9-Carboxy-11-nor-delta 9-THC, 1 mg/mL

14.3.3 Delta 9-THC-$d_3$, 100 µg/mL

14.3.4 9-Carboxy-11-nor-delta 9-THC-$d_3$, 1 mg/mL

14.3.5 N,O-bis(Trimethylsilyl)trifluoroacetamide with 1% Trimethylchlorosilane (BSTFA +1%TMCS)

14.3.6 Acetonitrile

14.3.7 Concentrated hydrochloric acid

14.3.8 Methanol

14.3.9 Hexane

14.3.10 Ethyl acetate

## 14.4 Solutions, Internal Standards, Calibrators, and Controls

14.4.1 0.1 N Hydrochloric Acid: Pipet 8.25 mL of concentrated hydrochloric acid into a 1 L volumetric flask and QS to volume with $dH_2O$.

14.4.2 Hexane/Ethyl Acetate (1:1): Mix 100 mL hexane with 100 mL ethyl acetate (v/v). Prepare fresh daily.

14.4.3 Calibrators

14.4.3.1 Working solution A (10 µg/mL): Pipet 100 µl each of THC and THCA stock solutions (1 mg/mL ) into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

14.4.3.2 Working solution B (1.0 µg/mL): Pipet 1 mL of working solution A into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

14.4.3.3 Working solution C (0.1 µg/mL): Pipet 1 mL working solution B into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

14.4.4 Controls

14.4.4.1 THC QC working solution A (10 µg/mL): Pipet 100 µL of THC stock solution (1 mg/mL) (*manufacturer or lot number different than that used for calibrators*) into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

14.4.4.2 QC solution (0.2 µg/mL THC and 2.0 µg/mL THCA): Pipet 200 µl of THC QC working solution A into a 10 mL volumetric flask. Add 20 µl of THCA stock solution (1 mg/mL). QS to volume with methanol. Store in freezer.

14.4.4.3 Control (0.004 mg/L THC and 0.040 mg/L THCA): Measure 20 µL of QC solution into an appropriately labeled 16 x 125 mm screw cap tube containing 1 mL blank blood.

14.4.4.4 Negative blood control: Blood bank blood or equivalent previously determined not to contain THC or THCA.

14.4.5 Internal Standard

14.4.5.1 Internal standard working solution A (10 µg/mL THC-$d_3$ and THCA-$d_3$): Pipet 1 mL of the THC-$d_3$ stock solution (100 µg/mL) and 100 µl of the THCA-$d_3$ stock solution (1 mg/mL) into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

14.4.5.2 Internal standard spiking solution (0.4 µg/mL THC-$d_3$ /THCA-$d_3$): Pipet 1 mL of the internal standard working solution A (10 µg/mL THC-$d_3$ /THCA-$d_3$) into a 25 mL volumetric flask and QS to volume with $dH_2O$. Prepare fresh daily.

14.4.6 Calibrators. Pipet the following volumes of working standards into 16 x 125 mm tubes to achieve the following calibrator concentrations.

14.4.6.1 Cal 1: 0.200 mg/L : 20 µL of 10 µg/mL working solution A

14.4.6.2 Cal 2: 0.100 mg/L: 10 µL of 10 µg/mL working solution A

14.4.6.3 Cal 3: 0.050 mg/L: 50 µL of 1.0 µg/mL working solution B

14.4.6.4 Cal 4: 0.010 mg/L: 10 µL of 1.0 µg/mL working solution B

14.4.6.5 Cal 5: 0.005 mg/L: 50 µL of 0.1 µg/mL working solution C

14.4.6.6 Cal 5: 0.002 mg/L: 20 µL of 0.1 µg/mL working solution C

14.4.6.7 Cal 5: 0.001 mg/L: 10 µL of 0.1 µg/mL working solution C

14.4.6.8 For each calibrator, evaporate standards to dryness under nitrogen and add 1 mL blank blood to each tube.

**14.5 Apparatus**

14.5.1 Agilent GC/MSD, Chemstation software, compatible computer and printer

14.5.2 Screw cap test tubes, 16 x 125 mm

14.5.3 Screw cap test tubes, 13 x 100 mm

14.5.4 100 x 13 mm disposable test tubes

14.5.5 Centrifuge capable of 2,000-3,000 rpm

14.5.6 Nitrogen evaporator with heating block

14.5.7 Vortex mixer

14.5.8 12 x 75 mm disposable test tubes

14.5.9 GC auto sampler vials with inserts

14.5.10 DPX-CSP-05 (Disposable Pipette Extraction tips from EST Analytical)

14.5.11 GC/MSD Conditions. Instrument conditions may be changed to permit improved performance.

14.5.11.1 Column: HP 5MS 25 m x 0.25 mm x 0.25 µm

14.5.11.2 Detector Temperature: 280° C

14.5.11.3 SIM parameters

| | |
| --- | --- |
| THC: | 386,371,303 |
| THC-$d_3$: | 374 |
| THCA: | 371,473,488 |
| THCA-$d_3$: | 374 |

14.5.11.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 110° C
- Initial time: 1 minutes
- Ramp: 10° C/min
- Final Temp: 290° C
- Final Time: 9 minutes
- Run Time: 28 minutes

14.5.11.5 Inlet

- Mode: Splitless
- Temperature: 270° C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

**14.6 Procedure**

14.6.1 Label clean 16 x 125 mm screw cap tubes appropriately, blank, calibrators, controls and case sample IDs.

14.6.2 Prepare calibrators and controls.

14.6.3 Add 1 mL case specimen to the appropriately labeled tubes.

14.6.4 Add 100 µL internal standard spiking solution to each tube. Vortex briefly.

14.6.5 Acetonitrile extraction

14.6.5.1 Add 2 mL acetonitrile to samples while vortexing.

14.6.5.2 Vortex an additional 20 seconds.

14.6.5.3 Centrifuge at 3000 rpm for 15 minutes.

14.6.5.4 Pour off acetonitrile layer into 13 x 100 mL test tube

14.6.5.5 Add 1 mL of 0.1 N hydrochloric acid to each sample extract

14.6.6 Extraction with DPX tips

14.6.6.1 Pre-wash the DPX tip with 500 µL hexane/ethyl acetate 1:1

14.6.6.2 Draw sample extract into tip (allowing air to mix solution and powder)

14.6.6.3 Let stand 20 seconds

14.6.6.4 Expel into original test tube

14.6.6.5 Redraw sample extract into tip

14.6.6.6 Let stand 20 seconds

14.6.6.7 Expel into original test tube

14.6.6.8 Draw 500 µL hexane/ethyl acetate 1:1 into tip

14.6.6.9 Let stand 20 seconds

14.6.6.10 Expel this first extract into empty 12 x 75 mm test tube

14.6.6.11 Draw another 500 µL hexane/ethyl acetate 1:1 into tip

14.6.6.12 Let stand 20 seconds

14.6.6.13 Expel this second extract into the 12 x 75 mm test tube containing the first extract

14.6.7 Derivitization

14.6.7.1 Remove water layer from bottom of combined extracts

14.6.7.2 Evaporate under nitrogen at 90° C

14.6.7.3 Add 40 µL ethyl acetate to test tubes and vortex

14.6.7.4 Transfer ethyl acetate to GC autosampler vials

14.6.7.5 Add 20 µL BSTFA +1% TCMS

14.6.7.6 Cap and inject 2 µL on GC/MSD in SIM mode

**14.7 Calculation**

14.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on peak heights (or areas) ratios (using the target ions underlined above) versus calibrator concentration.

14.7.2 Qualifier ion ratio range. The qualifier ion ratio range is calculated by determining the mean ion ratio from all calibrators used in the calibrations curve. Each drug has two qualifier ions.

**14.8 Quality Control**

14.8.1 See Toxicology Quality Guidelines

**14.9 References**

14.9.1 Rapid and Sensitive Analysis of THC and COOH-THC in Whole Blood. Brandi L. Clelland and William E. Brewer, Ph.D., DFTCB, SOFT presentation October 2001

14.9.2 Personal communiqué with William E. Brewer, Ph.D., January to August 2003

14.9.3 Dwight Flammia, Ph.D., Randall Edwards, and Terri Woods, in-house development.